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MICROSPORIDIUM ANOETI SP. N. (MICROSPORA) A NEW MICROSPORIDIAN PARASITE OF MITE *ANOETUS FERONIARUM* (ACARIFORMES, ANOETIDAE)

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***Microsporidium anoeti* sp. n. (Microspora) — новая микроспоридия из клеща *Anoetus feroniarum* (Acariformes, Anoetidae). Овчаренко Н., Золотарева Г., Вита И.** — На основании изучения ультраструктуры спор и доспоровых стадий описан новый вид микроспоридий из клеток паренхимы клеща *Anoetus feroniarum*. Характерными особенностями обнаруженных паразитов являются изолированные ядра на протяжении жизненного цикла и двуспоровая спорогония. Палочковидные споры микроспоридий имели размеры 0,7–0,9×2,2–2,4 μm (на полутонких срезах). Их оболочка состояла из екзоспори 18–25 nm и эндоспоры толщиной 30–40 nm. Характерными чертами строения аппарата экструзии спор являются изофиллярная полярная трубка, образующая спираль из 9–10 колець и пластинчастый поляропласт.
Ключевые слова: микроспоридии, *Microsporidium* , ультраструктура, Anoetidae, паренхима.

***Microsporidium anoeti* sp. n. (Microspora) a New Microsporidian Parasite of Mite *Anoetus feroniarum* (Acariformes, Anoetidae). Ovcharenko M., Zolotarjowa G., Wita.I.** — Microsporida were found for the first time in cytoplasm of parenchimatous cells of *Anoetidae* mites and described as a new species of *Microsporidium*. The final stages of merogony and disporoblastic sporogony with forming of uninucleate sporoblasts and spores were observed. The rod-shaped stained spores measured 0.7–0.9×2.2–2.4 μm. Uninucleate spores had 9–10 coils of isofilar polar filament and lamellar polaroplast, their thin envelope consisted of plasmalemma, 30–40 nm wide endospore and 18–25 nm wide exospore.
Key words: Microsporidia, *Microsporidium*, ultrastructure, Anoetidae, parenchimatous cells.

Introduction

Arthropods are considered one of the most common hosts for microsporidia, but there is no report of any microsporidium associated with Anoetidae. During hystological examination of laboratory colony of mites from potato we found a microsporidium located in the parenchymatous cells. We consider it a new species of the collectine group *Microsporidium* and present herein its ultrastructural characters.

Material and methods

The mites taken for hystological and ultrastructural investigations were fixed in 2.5% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) at 4 °C for 4th. After washing in cacodylate buffer and postfixation in 2.0% (w/v) osmium tetroxide in cacodylate buffer for 1 h at 4 °C, the mite was washed and dehydrated in an ascending series of ethanol to propylene oxide and embedded in Epon. Semithin sections were cut and stained with Toluidin Blue. Ultrathin sections were stained with uranyl acetate followed by lead citrate and observed in the JEM-100 B electron microscope.

Results

The microsporidium was found in one of the studied mites *Anoetus feroniarum* (Acariformes: Anoetidae) collected in December, 1996.

The earliest stages observed are interpreted as late merogonial plasmodia up to 1.5 μm in diameter (Fig. 1, *a*). The plasmodia with 1–3 nuclei divided into ameboid cells with a single nuclei 0.4–0.5 μm wide (Fig. 1, *b, c*). The sporogony was followed by binary fission and sporogonial stages were joined in chain-lake configurations. Each of the daughter cells was a sporoblast; its transformation into the spore followed the normal pattern of sporogenesis (Fig 1, *d*). The newly formed sporoblasts were irregularly rounded (Fig 1, *d*) but when they matured to spores, their shape changed to elongated and rod-like. The formed polar filament consisted of electron-dense layers surrounded by fine granular material (Fig 1, *e*).

Mature spores were uninucleate and uniform in shape and dimension, measuring 0.7–0.9 ×2.2–2.4 μm in semithin sections (Fig 2, *a*). Electron-transparent zone noticeable around the spores is interpreted as a zone of lisis, no parasitophorous vacuole was formed (Fig 2, *b*). Most of the spores were associated in chain-like disposition (Fig 2, *b, c*). Mature spores had lightly creased, electron-dense exospore measuring 18–25 nm in thickness. The electron-lucent endospore was 30–40 nm wide (Fig 2, *e*). The lamellar polaroplast occupied the anterior half of mature spore. The polar filament was of isofilar type, with 9–10 coils (Fig. 2, *d, e*).

In cross sections, the polar filament contained a central core surrounded by concentric layers of fine granular material. The angle of tilt of the polar filament coil to the long axis of the spore was 85-90 °C. The width of polar filament in cross sections was 90 nm, diameter of their basal part measured 110 nm. Structure of posterior vacuole remains unknown.

Discussion

Characteristic features of the described microsporidia are monocaryotic developmental stages, apansporoblastic and disporous sporogony and development in direct contact with the host cell cytoplasm.

Four microsporidial genera are known from acariform mites: *Nosema, Gurleya, Cryptosporina* and *Napamichum* (Spraque, 1977; Larsson, 1990). In contrast to the microsporidium we found *Nosema* — like species are diplokaryotic at all stages of the life cycle (Weiser, 1985; Larsson, 1988). Presence of sporophorous vesicles, polysporoblastic sporogony and diplocaryotic merogonial stages are characteristic features of the other mentioned genera. Microsporidia of the genus *Gurleya* have tetrasporoblastic sporogony and pyriform spores (Friedrich, et al., 1996). *Cryptosporina* — like microsporidia are octosporoblastic with amber-lake particles inside a very persistent sporophorous vesicle (Hazard, Oldacre, 1975). Octosporoblastic sporogony inside oval sporophorous vesicles, pyriform spores with layered exospore and anisofilar polar filament are characteristic features of the genus *Napamichum* (Larsson, 1990).

Among microsporidia described from acariform mites, only *Nosema steinchausi*, parasite of *Tyrophagus noxius* have "proliferation by binary fission" and oval to rod-shaped binuclear spores measuring 0.6–0.8×1.2–1.7 mm in stained condition (Weiser, 1956). In contrast, spores of the microsporidium we found were uninucleate.

Absence of sporophorous vesicle, life cycle with separated nuclei, isofilar polar filament and lamellar polaroplast are characteristic features of the genera *Unikaryon* Canning Lai et Lie, 1974 and *Canningia* Weiser et al, 1995. Type species of the latter genus, *Canningia spinidentis* from the fir bark beetle *Pityokteines spinidens* has short tubular spores with laterally inserted globular anchoring disc. On the contrary, the *Microsporidium*-like anchoring apparatus of microsporidium we found is mushroom-shaped and inserted apically.

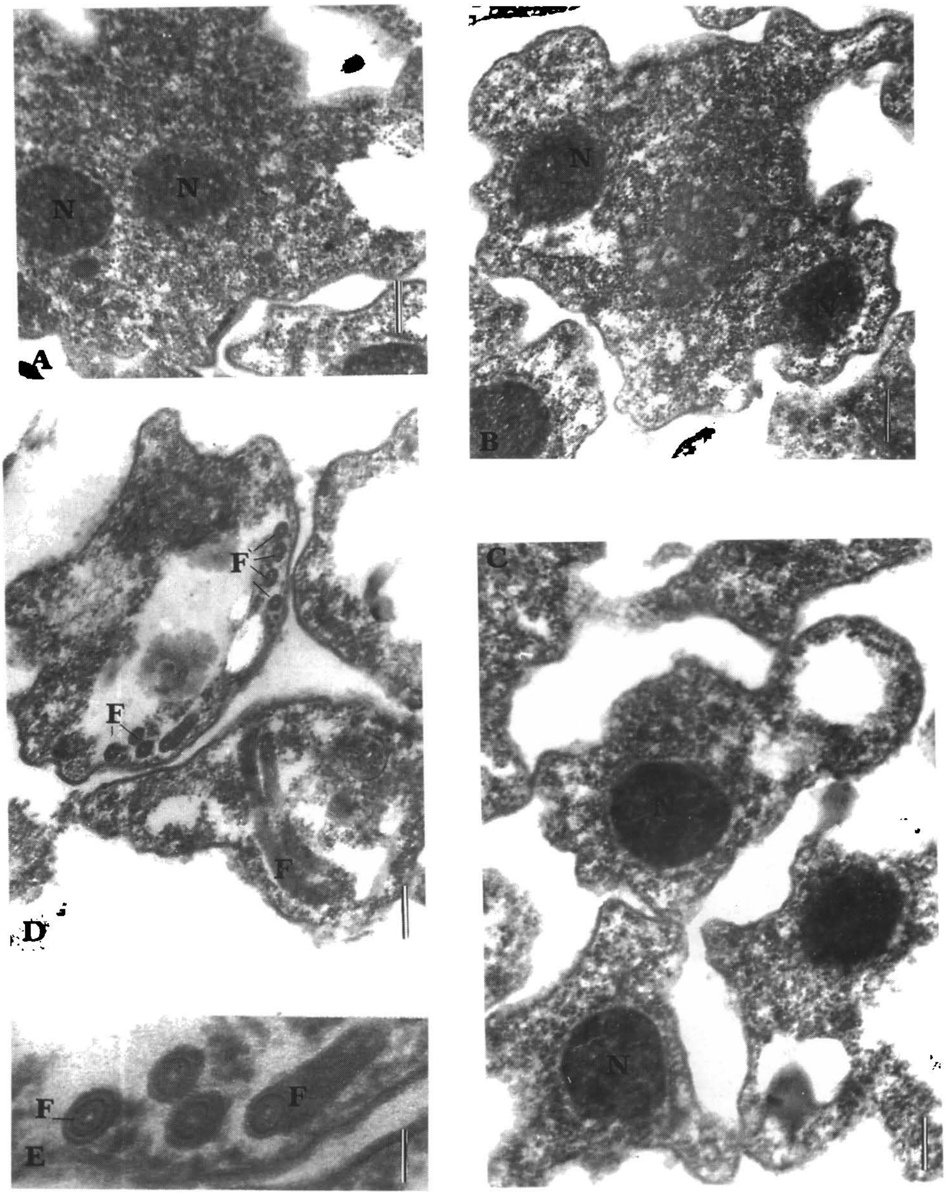


Fig. 1. Electron micrographs of presporal stages of microsporidia from *Anoetus feroniarum*. Late merogonial (A) and early sporogonial stages (B — C) with a rounded nuclei (N). D — group of sporoblasts with a polar filament coils (F). E — part of fig . 1—D. Transversal section of a multilayered polar filament (F). Bars: A — C = 200 nm, D = 250 nm, F = 80 nm.

The genus *Unikaryon* includes 9 species. Four of them — *U. pyriformis, U. legeri, U. allocreadi* and *U. slaptonleyi* — were described from *Trematoda* (Canning, Lai, Lie, 1974; Canning, Nicholas, 1974; Canning, Madhavi, 1977; Canning et al., 1983). *U. nomimoscolexi* was described from *Cestoda* (Sene, Ba, Marchand, Toguebaye, 1997). Three species (*U. bouixi, U. matteii, U. euzetii*) are know from *Coleoptera* and one (*U. mytilicolae)* from parasitic *Copepoda* (Toguebaye, Marchand, 1983, 1984, 1988; Durfort, Vallmitjana, Vivares, 1980).

The mentioned microsporidia have oval or pyriform spores up 2.9 to 5.0 µm long, while the microsporidium from *Anoetus feroniarum* were rod-shaped and smaller.

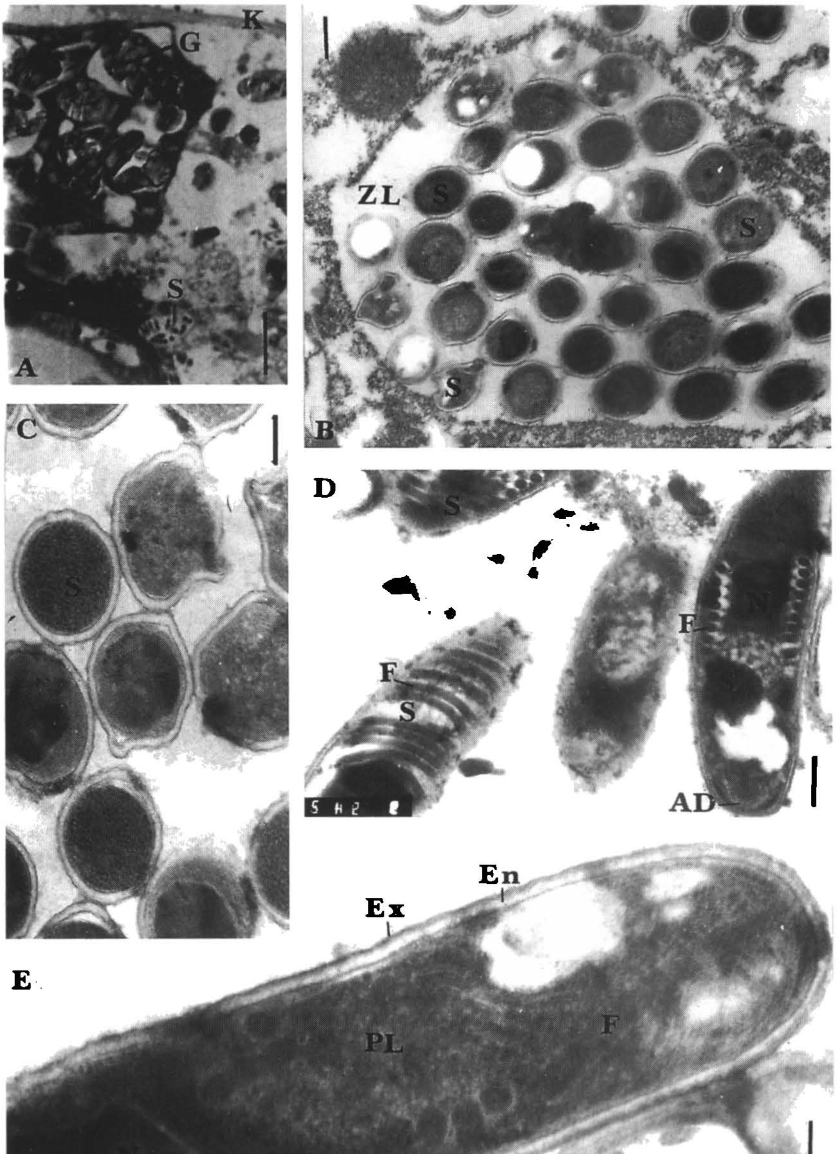


Fig. 2. Micrographs of mature spores. A — semithin section of *Anoetus feroniarum*. Microsporidian spores (S) are showed in a parenchim near cuticle (K) and guanin forming vesicle (G). B — Cytoplasm of a parenchimatous cell with a group of microsporidian spores (S) inside the lysis zone (ZL). C — chain—like disposition of spores in a transversal section. Longitudinal section of the spores with a coiled polar filament (F), a single nucleus (N) and anchoring disc (AD). E — anterior part of the spore. Details of the lamellar polaroplast (PL), polar filament (F) exospore (Ex) and endospore (En). Bars: A = 9.5 µm; B = 500 nm; C, D = 400 nm; E = 240 nm.

Microsporidium* *anoeti **sp. n.**

Host: *Anoetus feroniarum* (Acariformes: Anoetidae).

Life cycle: Final meronts and sporonts with a separate nuclei. Sporogony disporoblastic. Uninucleate spores rod-shaped measured 0.7—0.9×2.2—2.4 µm on stained semithin sections. Spores wall thin with 18—25 nm wide exospore and 30—40 nm wide

endospore. Polar filament 110–90 nm wide, arranged in 9–10 coils. The angle at tilt 85-90°. Polaroplast lamellar.

Type material on: slide N4701259; paratype on slide N4701260. Deposition of types in the collection of authors: Institute of Parasitology PAN, Warsaw, Poland, Institute of Hydrobiology NANU Kiev, Ukraine

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